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(54) Title: 1α-HYDROXY-24-*EPI*-VITAMIN D_4

(57) Abstract

1α-Hydroxy-24-*epi*-vitamin D_4 and novel intermediates formed in a novel method of preparing this compound. The method includes campesterol as a starting material which is converted to 24-*epi*-vitamin D_4 which is in turn hydroxylated to 1α-hydroxy-24-*epi*-vitamin D_4 via tosylated and cyclic derivatives of 24-*epi*-vitamin D_4. 1α-Hydroxy-24-*epi*-vitamin D_4 has been found to be bioactive.

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1α-HYDROXY-24-*EPI*-VITAMIN D_4

TECHNICAL FIELD

This invention relates to biologically active vitamin D_4 compounds. More specifically, this invention relates to novel 1α-hydroxy-24-*epi*-vitamin D_4 and a method for preparing this compound as well as novel intermediates formed in the synthesis.

BACKGROUND

The vitamins D are a group of compounds that are steroid derivatives and are known to be important in the regulation of calcium metabolism in animals and man. See, Harrison's Principles of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism, Chapter 335," E. Braunwald et al., (eds.), McGraw-Hill, New York, 1987, pp. 1860-1865.

The naturally occurring form of vitamin D in animals and man is vitamin D_3. Vitamin D_3 is synthesized endogenously in the skin of animals and man. In animals, including man, vitamin D_3 is activated by being hydroxylated in the C_{25} position in the liver, followed by 1α-hydroxylation in the kidney to produce the hormone 1α,25-dihydroxy vitamin D_3. See, U.S. Patent No. 3,880,894.

1α,25-Dihydroxy vitamin D_3 is the hormonally active form of vitamin D_3. This hormone is taken up in the intestine by specific cytoplasmic receptor proteins to stimulate calcium and phosphate transport from the intestinal lumen to circulation. The vitamin D_3 hormone also is taken up by specific cytoplasmic receptors in the parathyroid glands, the kidney, the osteoblasts, and other target tissues, to elicit cellular responses

which, synergistically, stabilize blood levels of calcium and phosphorus, control the formation and removal of bone, and regulate the further production of 1α,25-dihydroxy vitamin D_3 itself. It is now recognized that the 1α-hydroxy group is important in the binding of 1α,25-dihydroxy vitamin D_3 with its specific cytoplasmic receptors. It has also recently been reported that the vitamin D_3 hormones may play a role in cell proliferation and differentiation.

Vitamin D_2 is the major, naturally occurring form of vitamin D found in plants. Vitamin D_2 differs structurally from vitamin D_3 in that vitamin D_2 has a methyl group at C_{24} and has a double bond between C_{22} and C_{23}.

Considerable interest has focused on discovery and synthesis of various hydroxylated and dihydroxylated derivatives of vitamins D_3 and D_2. Examples of hydroxylated and dihydroxylated metabolites of vitamins D_3 and D_2 which have been found to occur naturally and/or have been synthesized include 25-hydroxy vitamin D_2, 24, 25-dihydroxy vitamin D_3, 25, 26-dihydroxy vitamin D_3, 1α-hydroxy vitamin D_2, 23, 25-dihydroxy vitamin D_3, all of which have been found to exhibit vitamin D-like biological activity <u>in vivo</u>.

Unfortunately, while many of these active vitamin D metabolites held great promise as therapeutic agents, this promise has never been fully realized because of the extreme toxicity of these agents. For example, toxicity limits the efficacy of vitamin D_3, its active forms and analogs, to prevent bone loss or restore lost bone. Many studies indicate that at dosages required for these agents to be effective in bone loss prevention or restoration, hypercalcemia and hypercalciuria are serious problems. It has been reported that 1α-hydroxy vitamin D_3 at a daily dose of 2 μg/day (which has been shown in some studies to be effective in preventing loss of bone) causes toxicity in approximately 67 percent of patients.

Vitamin D_4 is a little known form of vitamin D. Vitamin D_4 was first described in 1936. See, Grab, W., Z. Physiol. Chem., 243:63 (1936); McDonald, F. G., J. Biol. Chem., 114:IVX (1936). See also, Windaus, A. and Trautmann, G., Z. Physiol. Chem., 247:185-188 (1937). Vitamin D_4, also known as irradiated 22,23-dihydro-ergosterol or 22,23-dihydro vitamin D_2 or 22,23-dihydroergocalciferol, differs from vitamin D_3 in that it contains a C_{24} methyl group. The above cited references disagree as to the level of biological activity of this D vitamin, suggesting that in the rat, vitamin D_4 is one-third or three-fourths as active as vitamin D_3, and in the chick, either one-tenth or one-fifth as active as vitamin D_3.

In 1968, DeLuca et al. (Arch. Biochem. Biophys., 124:122-128 (1968)) confirmed that vitamin D_4 was less active than vitamin D_3. DeLuca et al. reported that vitamin D_4 is two-thirds as active as vitamin D_3 or vitamin D_2 in the rat, and one-fifth as active as vitamin D_3 in the chick.

DeLuca et al. make reference to the fact that "[t]he synthesis of vitamin D_4 has apparently been little used since it was first described by Windaus and Trautmann," and comment, "[t]his is perhaps due to the fact that vitamin D_4 is only of academic interest."

To applicants' knowledge, vitamin D_4 has remained "only of academic interest" as applicants are unaware of any further study of vitamin D_4 since that reported by DeLuca et. al. In fact, The Merck Index states with respect to vitamin D_4, "[i]ts biological activity seems doubtful." Merck Index, S. Budavari (ed.), 11th ed., Merck & Co., Rahway, N.J., (1989) pp. 1579, #9930.

There has been even less interest in vitamin D_4 analogues. Recently, however, a vitamin D_4 analogue, 1α-hydroxy vitamin D_4, has been synthesized and shown to possess unexpectedly high biopotency and low toxicity (co-pending U.S. Patent Application Serial

No. 07/586,854, filed September 21, 1990). It was surprising to applicants in that application that this vitamin D_4 analogue had activity commensurate with the vitamin D_3 and D_2 hormones. Applicants, in this invention, have synthesized a related isomer of 1α-hydroxy vitamin D_4 with equally surprising biological activity.

SUMMARY OF THE INVENTION

The present invention provides a stereoisomer of vitamin D_4, 1α-hydroxy-24-*epi*-vitamin D_4, tosylated and cyclic derivatives of this compound, and a method of preparing these compounds.

In one aspect, the invention provides the compounds of formula (I) as defined hereinbelow. 1α-Hydroxy-24-*epi*-vitamin D_4, the compound of formula (I) wherein R_1 and R_2 are each hydroxy groups, has been found to be bioactive. Other compounds encompassed by formula (I) have been found to be novel intermediates in the synthesis of 1α-hydroxy-24-*epi*-vitamin D_4.

In another aspect, the invention provides the compounds of formula (II) which have also been found to be novel intermediates in the synthesis of 1α-hydroxy-24-*epi*-vitamin D_4.

In further aspect, the invention provides a synthetic route for making the 1α-hydroxy-24-*epi*-vitamin D_4. The method includes campesterol as a starting material which is converted to 24-*epi*-vitamin D_4 which is in turn hydroxylated to 1α-hydroxy-24-*epi*-vitamin D_4 via tosylated and cyclic derivatives of 24-*epi*-vitamin D_4. A novel intermediate which is a derivative of campesterol has also been found.

Other advantages and a fuller appreciation of the specific adaptations, compositional variations, and physical and chemical attributes of the present invention will be gained upon an examination of the following detailed description of the invention, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinafter be described in conjunction with the appended drawings, wherein like designations refer to like elements throughout and in which:

Figure 1 illustrates preparative steps for the synthesis of 24-*epi*-vitamin D_4 starting with campesterol; and

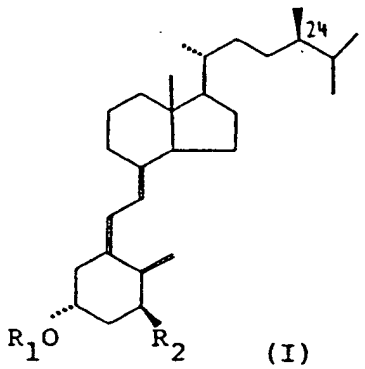
Figure 2 illustrates preparative steps for the synthesis of 1α-hydroxy-24-*epi*-vitamin D_4 starting with 24-*epi*-vitamin D_4.

DETAILED DESCRIPTION

The present invention provides synthetic 1α-hydroxy-24-*epi*-vitamin D_4 (1α-OH-24-*epi*-D_4) as well as tosylated and cyclic derivatives of this compound.

As used herein, the terms "biological activity" or "biologically active" are meant to refer to biochemical properties of compounds such as affecting metabolism, e.g., affecting serum calcium concentration, or binding to an appropriate receptor protein, e.g., binding to vitamin D receptor protein. The term "*epi*" as used herein and as used generally in the art is meant to designate a different absolute configuration about a carbon atom, in the present invention, about the C_{24} carbon, than in the parent vitamin D_4 structure.

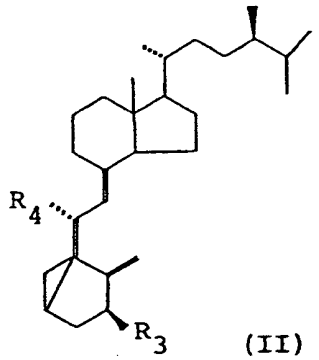
In one of its aspects, the invention encompasses the compounds of the general formula (I):



(I)

wherein R_1 is hydrogen or tosyl and R_2 is hydrogen or hydroxy, and salts, hydrates and solvates thereof. Preferred among those compounds of formula (I) is that in which R_1 is hydrogen and R_2 is OH, i.e., 1α-hydroxy-24-*epi*-vitamin D_4, which has been found to increase serum calcium.

In another aspect, the invention provides compounds of formula (II):



(II)

wherein R_3 is either hydrogen or hydroxy, and R_4 is methoxy, and salts, hydrates and solvates thereof. These compounds have been found to be useful and novel intermediates to form 1α-hydroxy-24-*epi*-vitamin D_4.

In still another aspect, the invention involves the preparation of compounds of formulas (I) and (II) as well as another novel intermediate. Specifically, the synthesis of 1α-hydroxy-24-*epi*-vitamin D_4, i.e., the compound of formula (I) wherein R_1 is hydrogen and R_2 is OH, is accomplished according to the schema presented in Figures 1 and 2. As seen in Figure 1, the synthesis uses the steroid campesterol as the starting material. Campesterol is available according to the procedure of Tarzia et al., Gazz. Chem. Ital., vol. 97, pp. 102-106 (1967). Campesterol undergoes C_7 bromination, C_7-C_8 dehydrobromination in a four-step process to yield 7-dehydrocampersterol. The 7-dehydrocampesterol is then irradiated and thermally converted by methods well known

in the art to yield 24-*epi*-vitamin D_4 [also known as 22,23-dihydro-24-*epi*-ergocalciferol]. As seen in Figure 2, 24-*epi*-vitamin D_4 is then hydroxylated in a five-step process to yield 1α-hydroxy-24-*epi*-vitamin D_4.

Specifically, campesterol is acetylated to form the 3β-acetate. This campesterol acetate is subjected to C_7 bromination, C_7-C_8 dehydrobromination to form a double bond at C_7-C_8. The resulting 7-dehydrocampesterol acetate is then reduced to the novel 7-dehydrocampesterol. The 7-dehydrocampesterol is then irradiated and thermally converted to yield 24-*epi*-vitamin D_4. The 24-*epi*-vitamin D_4 is then tosylated to yield the 3β-tosylate of 24-*epi*-vitamin D_4. The tosylate is displaced by solvolysis to yield the 6-methoxylate of 24-*epi*-3,5-cyclovitamin D_4. This 24-*epi*-cyclovitamin D_4 is subjected to allylic oxidation to form the 1α-hydroxy 24-*epi*-cyclovitamin derivative. The 1α-hydroxy 24-*epi*-cyclovitamin derivative is sequentially hydrolyzed and subjected to a Diels-Alder type reaction which removes the 6-methoxy group and separates the 1α-hydroxy 24-*epi*-vitamin D_4 (5,6 cis) from the 5,6 trans 1α-hydroxy 24-*epi*-vitamin D_4. It is noted that the trans isomer, if desired, may be separated from the cis isomer via high pressure liquid chromatography according to the procedure disclosed, for example, in U.S. Patent 4,719,204 issued to DeLuca et al.

1α-Hydroxy-24-*epi*-vitamin D_4 has been found to possess physiological activity, namely, as an agent for increasing serum calcium concentrations. Specifically, this compound increases serum calcium concentrations in rats with vitamin D deficiency. Thus, the compounds of the invention are potentially applicable to various clinical and veterinary fields, and are particularly useful for the treatment of abnormal metabolism of calcium and phosphorus.

The following examples are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the

following examples, all temperatures are set forth in degrees Celsius; unless otherwise indicated, all product yields are reported as percentages by weight. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker AM--400(400 MHz) with aspect 3000 Computer in $CDCl_3$ solutions with $CHCl_3$ as an internal standard. Chemical shifts are reported in ppm. Ultraviolet spectra were recorded with a Hitachi U-2000 Spectrophotometer and are reported for ethanol solutions.

Example 1: Synthesis of 1α-hydroxy-24-*epi*-vitamin D_4

Campesterol Acetate (2):

To a solution of 24.0 g (0.06 mol) of campesterol (1) in 180 ml of anhydrous pyridine was added 18.5 ml (0.196 mol) of acetic anhydride. The mixture was stirred at room temperature overnight and then 600 ml of water was added. The precipitate was filtered and washed three times with 200 ml portions of acetonitrile, and then air dried to yield 20.0 g (75%) of (2).

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.7 (3H, s, 18-CH_3), 0.8 (6H, dd, 26 and 27-CH_3), 0.86 (3H, d, 21-CH_3), 0.92 (3H, d, 28-CH_3), 1.02 (3H, s, 19-CH_3), 2.04 (3H, s, $OCOCH_3$), 4.6 (1H, m, 3-H), 5.38 (1H, m, 6-H).

7-Dehydrocampesterol acetate (3)

A mixture of 10 g (0.023 mol) of (2), 4.56 g (0.016 mol) of dibromantin and 10.2 g (0.121 mol) of anhydrous sodium bicarbonate in 250 ml of dry hexane was heated under reflux in a nitrogen atmosphere for 2 hrs. The precipitate was filtered off and the solution was concentrated to dryness under reduced pressure. To the solution of the residue in 50 ml of anhydrous tetrahydrofuran was added 0.65 g (2.02 mmol) of tetrabutylammonium bromide, and the mixture was stirred at room temperature for 30 min under nitrogen. A solution of tetrabutylammonium fluoride (112 ml, 1M in

THF) was then added followed by 5.0 ml of s-collidine, and the mixture was stirred under nitrogen at room temperature overnight. To this reaction mixture was added ether (700 ml), and the organic phase was washed with water (2x200 ml), cold 1M HCl (2x200 ml) and 10% sodium bicarbonate (2x200 ml), and dried over anhydrous $MgSO_4$. Chromatography on silica gel with 10% ethyl acetate in hexane gave 5.5 g (55%) of (3).

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.62 (3H, s, 18-CH_3), 0.80 (6H, dd, 26 and 27-CH_3), 0.86 (3H, d, 21-CH_3), 0.94 (3H, d, 28-CH_3), 0.96 (3H, s, 19-CH_3), 2.05 (3H, s, $OCOCH_3$), 4.7 (1H, m 3-H), 5.4 (1H, m, 7-H), 5.58 (1H, m, 6-H).

7-Dehydrocampesterol (4)

To a solution of 5.5 g (0.012 mol) of (3) in dry ether (500 ml) was added 3.38 g (0.089 mol) of lithium aluminum hydride. The mixture was stirred at room temperature for 2 hours, cooled with an ice water bath, and the reaction mixture decomposed by the cautious dropwise addition of ice water (5 ml). The mixture was filtered and the filtrate was concentrated *in vacuo* to remove most of the tetrahydrofuran. The residue was dissolved in 1000 ml of ether and washed with saturated NaCl solution (2x500 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column using 20% ethyl acetate in hexane to yield 4.0 g (80%) of (4).

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.62 (3H, s, 18-CH_3), 0.8 (6H, dd, 26 and 27-CH_3), 0.86 (3H, d, 21-CH_3), 0.94 (3H, d, 28-CH_3), 0.96 (3H, s, 19-CH_3), 3.62 (1H, m, 3-H), 5.39 (1H, m, 7-H), 5.58 (1H, m, 6-H).

24-*epi*-Vitamin D_4 (5)

7-Dehydrocampesterol (4) (3.0 g, 7.5 mmol) was dissolved in 500 ml of ether and benzene (4:1) and irradiated with stirring under nitrogen in a water-cooled quartz immersion well using a Hanovia

medium-pressure UV lamp for 1.5 hrs. The solution was concentrated *in vacuo*, redissolved in 200 ml of ethanol and heated under reflux overnight. The solution was concentrated to dryness *in vacuo* and the residue was purified on a silica gel column using 20% ethyl acetate in hexane to yield 0.9 g (30%) of (5).

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.54 (3H, s, 18-CH_3), 0.76 (6H, dd, 26 and 27-CH_3), 0.82 (3H, d, 21-CH_3), 0.9 (3H, d, 28-CH_3), 3.91 (1H, m, 3-H), 4.7 (1H, m, 19-H), 5.03 (1H, m, 19-H), 6.02 (1H, d, 7-H), 6.21 (1H, d, 6-H). UV (ethanol) λ_{max}: 265 nm.

24-*epi* Vitamin-D_4 tosylate (6)

To a solution of 0.9 g (2.26 mmol) of (5) dissolved in 10 ml of anhydrous pyridine was added 1.2 g (6.30 mmol) of tosyl chloride. The mixture was stirred under nitrogen at 5°C for 24 hrs. The reaction mixture was poured into 100 ml of cold saturated $NaHCO_3$ solution and extracted with ether (3x200 ml). The combined ether extracts were washed with 5% HCl solution (3x300 ml), saturated sodium bicarbonate solution (3x300 ml) and saturated NaCl solution (2x300 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield 1.1 g (88%) of (6).

24-*epi*-3,5-Cyclovitamin D_4 (7)

To a solution of 1.0 g (1.81 mmol) of (6) dissolved in 100 ml of anhydrous methanol was added sodium bicarbonate 10.0 g (0.12 mol). The mixture was heated under reflux for 8 hrs. The reaction mixture was concentrated *in vacuo*. Water (200 ml) was added followed by extraction with ether (2x300 ml). The combined ether extracts were dried over anhydrous $MgSO_4$ and concentrated to dryness *in vacuo* to yield 600 mg (80%) of (7) as an oil.

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.54 (3H, s, 18-CH_3), 0.78 (6H, dd, 26 and 27-CH_3), 0.86 (3H, d, 21-CH_3), 0.92 (3H, d, 28-CH_3), 3.25 (3H, s, -OCH_3), 4.16 (1H, d, 6-H),

4.86 (1H, m, 19-H), 4.98 (1H, d, 7-H), 5.02 (1H, m, 19-H).

1α-Hydroxy-24-epi-3,5-cyclovitamin D_4 (8)

tert-Butyl hydroperoxide (1.13 ml, 3.39 mmol; 3M in toluene) was added to a suspension of 95 mg (0.86 mmol) of selenium dioxide in 65 ml of anhydrous dichloromethane under nitrogen. The mixture was stirred at room temperature under nitrogen for 3 hours. Then 0.13 ml of anhydrous pyridine was added followed by a solution of 600 mg (1.45 mmol) of (7) dissolved in 20 ml of anhydrous dichloromethane. The mixture was stirred under nitrogen at room temperature for 15 min, then 25 ml of 10% NaOH solution was added and the mixture was extracted with ether (3x100 ml). The combined ether extracts were washed with 10% NaOH solution (3x100 ml), water (3x100 ml), saturated sodium chloride solution (2x100 ml), dried over anhydrous $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was purified on a silica gel column using a mixture of 20% ethyl acetate in hexane to yield 140 mg (23%) of (8) as an oil.

^{1}H NMR: (400 MHz, $CDCl_3$); δppm, 0.54 (3H, s, 18-CH_3), 0.79 (6H, dd, 26 and 27-CH_3), 0.88 (3H, d, 21-CH_3), 0.92 (3H, d, 28-CH_3), 3.24 (3H, s, -OCH_3) 4.2 (1H, m, 3-H), 4.21 (1H, d, 6-H), 4.94 (1H, d, 7-H), 5.15 (1H, m, 19-H), 5.21 (1H, m, 19-H).

5,6-*cis* and 5,6-*trans*-1α-hydroxy-24-*epi*-vitamin D_4 (9, 10)

1α-Hydroxy-24-*epi*-3,5 cyclovitamin D_4 (8) (110 mg, 0.26 mmol) was dissolved in 1.1 ml of dimethylsulfoxide and 0.9 ml of acetic acid and heated at 50°C under nitrogen for 1 hour. The solution was poured over ice and 50 ml of saturated $NaHCO_3$ solution. The mixture was extracted with ether (3x100 ml). The combined ether extracts were washed with saturated $NaHCO_3$ solution (3x100 ml), water (2x100 ml), and saturated NaCl solution (2x200 ml), dried over anhydrous $MgSO_4$ and

concentrated *in vacuo* to yield the crude product 105 mg (95%) of (9) and (10).

5,6-*cis*-1α-hydroxy-24-*epi*-vitamin D_4 (9)

To a solution of (9) and (10), 105 mg (0.25 mmol) in 5 ml of ethyl acetate, was added 20 mg (0.2 mmol) of maleic anhydride, and the mixture was stirred at 35°C for 24 hours under nitrogen. The solution was concentrated to dryness *in vacuo*. The residue was purified on a silica gel column using 40% ethyl acetate in hexane to yield 30 mg (28%) of (9).

^{1}H NMR: (400 MHz, $CDCl_3$); δppm 0.54 (3H, 1 s, 18-CH_3), 0.78 (6H, dd, 26 and 27-CH_3), 0.86 (3H, d, 21-CH_3), 0.92 (3H, d, 28-CH_3), 4.2 (1H, m, 3-H), 4.41 (1H, m, 1-H), 5.0 (1H, m, 19-H), 5.32 (1H, m, 19-H), 6.0 (1H, m, 7-H), 6.38 (1H, m, 6-H); UV (ethanol) λ_{max}: 265 nm.

Example 2: Biological testing of 1α-hydroxy-24-*epi*-vitamin D_4

Male weanling rats (Holtzman strain, Holtzman Company, Madison, Wisconsin) were fed a vitamin D deficient diet containing adequate calcium (0.47%) and phosphorus (0.3%). Within three to four weeks, this diet induces an extreme vitamin D deficiency characterized by low serum calcium and poor growth. After four weeks on this diet, the rats had serum calcium values less than 6 mg/dl. The rats were then separated into four groups and orally administered either 1α-hydroxy-24-*epi*-vitamin D_4 in a vehicle such as coconut oil or the vehicle (control) for each of 14 days. Twenty-four hours after the last dose, the rats were killed, and the blood calcium measured by a standard laboratory technique. The results of these determinations are shown in Table 1.

Table 1

Increase in serum calcium concentration

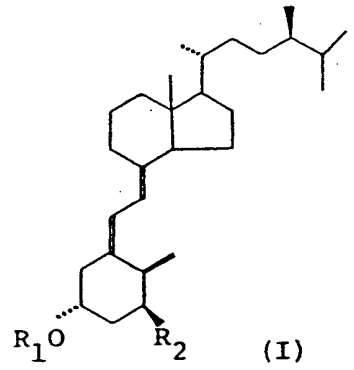
Compound	Dose (mcg/kg/day)	Number of Rats	Serum Calcium Concentration (mg/100 ml) ± Standard Deviation
Vehicle	-	10	5.1 ± 0.42
24-*epi*-1α-OH-D_4	0.042	11	5.8 ± 0.40
24-*epi*-1α-OH-D_4	0.250	12	8.1 ± 1.25
24-*epi*-1α-OH-D_4	1.500	12	10.5 ± 0.71

The data of Table 1 indicate that 1α-hydroxy-24-*epi*-vitamin D_4 is effective at increasing serum calcium in the vitamin D deficient rat and that the response appears to be dose dependent.

While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions, and omissions, that may be made in what has been described. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely by the broadest interpretation that lawfully can be accorded the appended claims.

CLAIMS:

1. The compound of the formula (I):



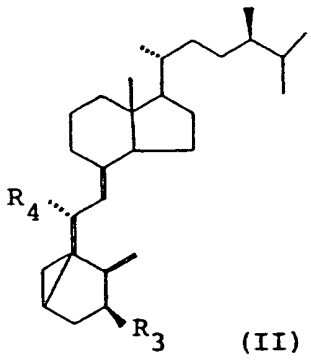
(I)

wherein R_1 is either hydrogen or tosyl and R_2 is either hydrogen or hydroxy, and salts, hydrates and solvates thereof.

2. The compound of claim 1, wherein the compound is 1α-hydroxy-24-*epi*-vitamin D_4.

3. The compound of claim 1, wherein the compound is 24-*epi*-vitamin D_4 tosylate.

4. The compound of the formula (II):



(II)

wherein R_3 is hydrogen or hydroxy and R_4 is methoxy.

5. The compound of claim 4, wherein the compound is 24-*epi*-3,5-cyclovitamin D_4.

6. The compound of claim 4, wherein the compound is 1α-hydroxy-24-*epi*-3,5-cyclovitamin D_4.

7. 7-Dehydrocampesterol.

8. 5,6-trans-1α-hydroxy-24-*epi*-vitamin D_4.

9. A method of preparing 1α-hydroxy-24-*epi*-vitamin D_4, comprising:

(a) tosylating 24-*epi*-vitamin D_4 in the presence of dry pyridine to form 24-*epi*-vitamin D_4 tosylate;

(b) solvolyzing 24-*epi*-vitamin D_4 tosylate to form 24-*epi*-3,5 cyclovitamin D_4;

(c) allylically oxidizing the 24-*epi*-3,5 cyclovitamin D_4 with selenium dioxide to form 1α-hydroxy-24-*epi*-3,5-cyclovitamin D_4; and

(d) hydrolyzing the 1α-hydroxy-24-*epi*-3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy-24-*epi*-vitamin D_4 and 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 and forming a Diels-Alder adduct of the 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 to allow purification to yield 1α-hydroxy-24-*epi*-vitamin D_4.

10. A method of producing 24-*epi*-vitamin D_4 tosylate, comprising reacting 24-*epi*-vitamin D_4 with toluenesulfonyl chloride in the presence of dry pyridine.

11. A method of producing 24-*epi*-3,5-cyclovitamin D_4, comprising subjecting 24-*epi*-vitamin D_4 tosylate to buffered solvolysis.

12. A method of producing 1α-hydroxy-24-*epi*-3,5-cyclovitamin D_4, comprising allylically oxidizing the 24-*epi*-3,5-cyclovitamin D_4 with selenium dioxide.

13. A method of producing 1α-hydroxy-24-*epi*-vitamin D_4, comprising hydrolyzing the 1α-hydroxy-24-*epi*-3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy-24-*epi*-vitamin D_4 and 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 and subjecting the admixture to a Diels-Alder reaction forming an adduct of the 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 to allow purification to yield the 1α-hydroxy-24-*epi*-vitamin D_4.

14. A method of producing 1α-hydroxy-24-*epi*-vitamin D_4, comprising: oxidizing campesterol to form 7-dehydrocampesterol; irradiating the 7-dehydrocampesterol to form 24-*epi*-vitamin D_4; and hydroxylating 24-*epi*-vitamin D_4 to form 1α-hydroxy-24-*epi*-vitamin D_4.

15. A method of producing 1α-hydroxy-24-*epi*-vitamin D_4, comprising:

(a) acetylating campesterol to form campesterol acetate;

(b) oxidizing the campesterol acetate to form 7-dehydrocampesterol acetate;

(c) reducing the 7-dehydrocampesterol acetate to 7-dehydrocampesterol;

(d) irradiating and thermally converting 7-dehydrocampesterol to form 24-*epi*-vitamin D_4;

(e) tosylating 24-*epi*-vitamin D_4 in the presence of dry pyridine to form 24-*epi*-vitamin D_4 tosylate;

(f) solvolyzing 24-*epi*-vitamin D_4 tosylate to form 24-*epi*-3,5-cyclovitamin D_4;

(g) allylically oxidizing the 24-*epi*-3,5-cyclovitamin D_4 with selenium dioxide to form 1α-hydroxy-24-*epi*-3,5-cyclovitamin D_4; and

(h) hydrolyzing the 1α-hydroxy-24-*epi*-3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy-24-*epi*-vitamin D_4 and 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 and forming a Diels-Alder adduct of the 5,6 trans 1α-hydroxy-24-*epi*-vitamin D_4 to allow purification to yield 1α-hydroxy-24-*epi*-vitamin D_4.

FIGURE 1

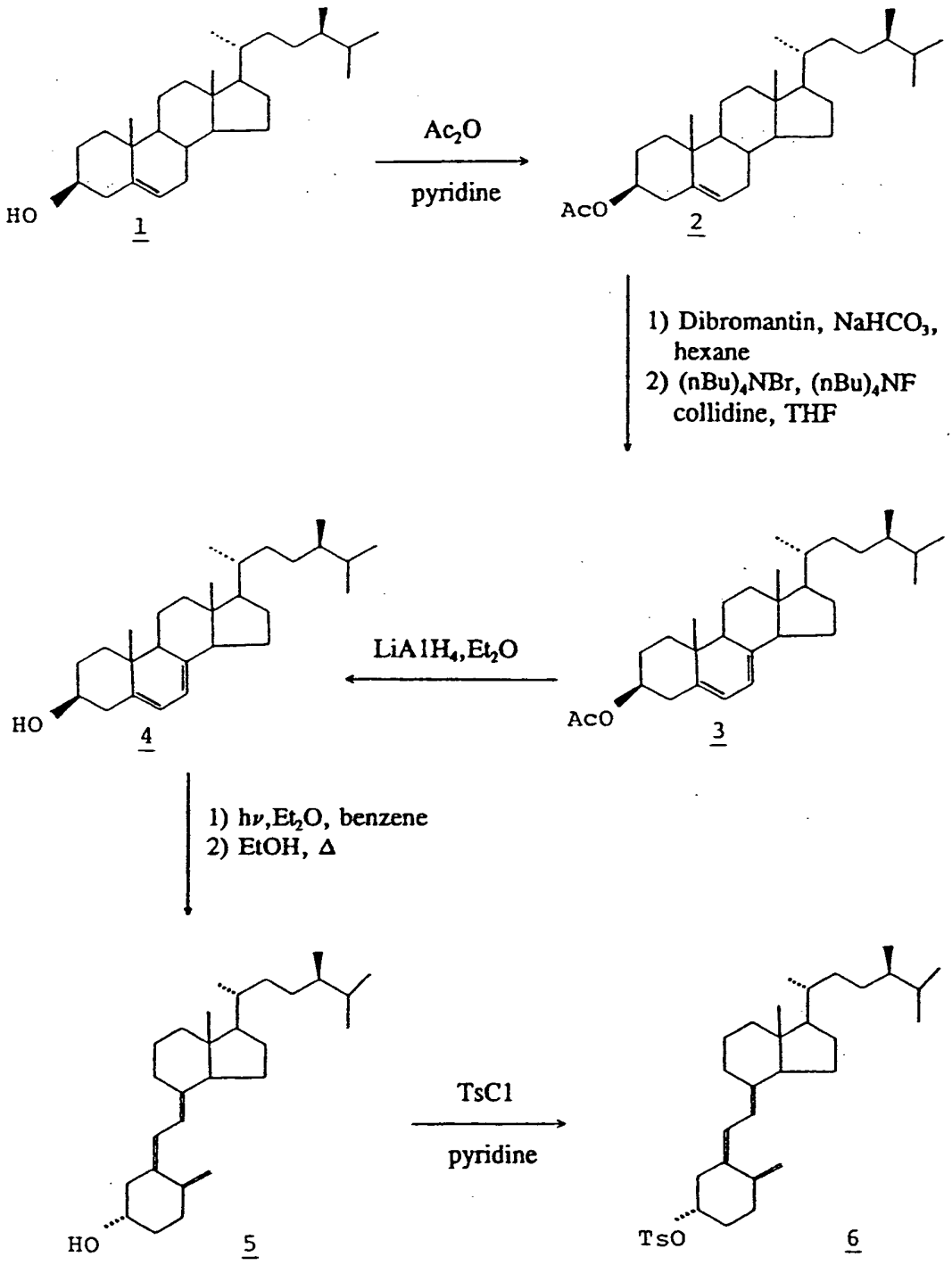
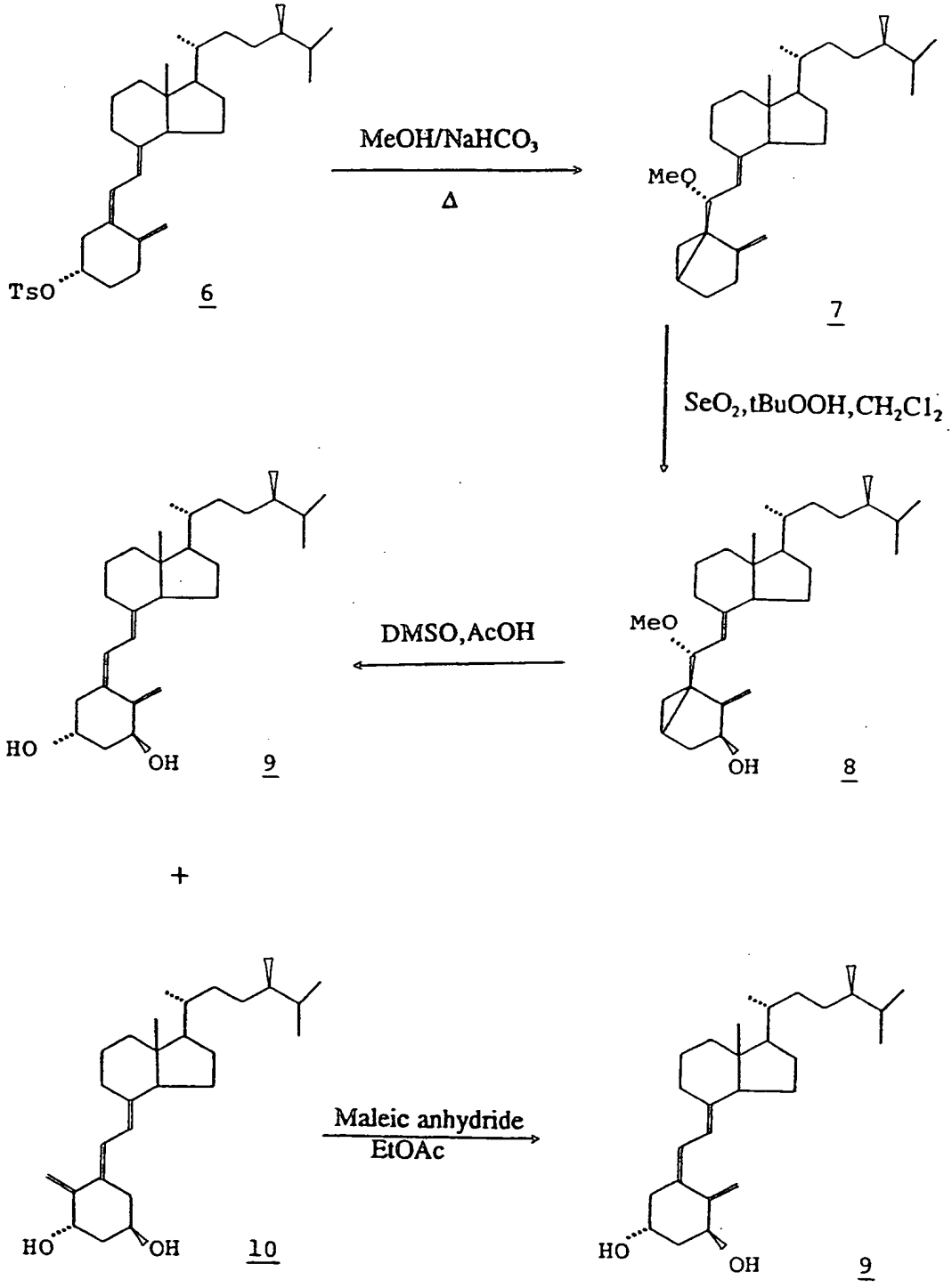

HO
1
Ac2O
pyridine
AcO
2
1) Dibromantin, NaHCO3,
hexane
2) (nBu)4NBr, (nBu)4NF
collidine, THF
HO
4
LiAlH4,Et2O
AcO
3
1) hν,Et2O, benzene
2) EtOH, Δ
HO
5
TsCl
pyridine
TsO
6

FIGURE 2


MeOH/NaHCO3
Δ
TsO
6
MeO
7
SeO2,tBuOOH,CH2Cl2
DMSO,AcOH
HO
OH
9
MeO
OH
8
+
HO
OH
10
Maleic anhydride
EtOAc
HO
OH
9

INTERNATIC L SEARCH REPORT

.ernational application No.
PCT/US93/00796

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/59 C07C 403/00

US CL :552/653; 514/167

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,769,181 (DeLUCA, ET AL.) 06 September 1988, See entire document.	1-6,8
Y	US, A, 4,973,584 (DeLUCA, ET AL.) 27 November 1990, See entire document.	1-6,8
Y	Archives of Biochemistry and Biophysics, 1968, (DeLUCA, ET AL.) "Synthesis, Biological Activity, and Metabolism of 22,23-H-Vitamin D4", pp. 122-128, esp. 122, 125-28.	1-8
Y	US, A, 4,448,721 (DeLUCA, ET AL.) 15 May 1984, See entire document.	8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
04 MAY 1993	09 JUN 1993

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